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DERMAL UPTAKE OF DILUTE AQUEOUS TRICHLOROETHYLENE BY HAIRLESS GUINEA PIGS

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TECHNICAL REVIEW AND APPROVAL

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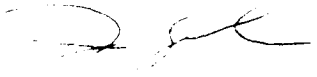
The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER



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Percutaneous absorption was measured in Na-pentobarbital-sedated female hairless guinea pigs dermally exposed for 70 min to ^{14}C-labeled trichloroethylene (TCE) present at either low (20- to 110-ppb) or high (100,000-ppb) concentrations in water at 32°. A glass dermal exposure chamber was used with an aluminum-coated latex diaphragm to obtain a continuous and completely aqueous exposure of approximately 75% of the surface area of each sedated animal. Dermal permeability constants for TCE (in liters of water cleared of TCE per cm^2-hr of exposure) were estimated for low- and high-concentration animal-exposure experiments based on the measured rate of radiolabel loss from chamber water after controlling for the amount of such loss observed in control exposures in which a glass beaker was used in place of a live animal. Uptake was verified by measurement of radiolabel in urine and feces for up to 4 weeks postexposure. The average permeability constant for extremely dilute aqueous TCE obtained from 5 animal experiments using this dermal exposure system was 1.4×10^{-4} liters cm^2-hr; this value was not significantly different from the corresponding mean value obtained from 5 animal experiments conducted using 100,000 ppb aqueous TCE. The observed clearance of ^{14}C-TCE from chamber water was approximately linear over the dermal exposure period. These are the first reported data on in vivo (continued on reverse)

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dermal absorption of a compound in extremely dilute aqueous solution.

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Ingestion of drinking water containing volatile organic compounds (VOCs) that are suspect human carcinogens is generally considered a potentially significant source of cancer risk for the U.S. population (Crouch et al., 1983; U.S. EPA, 1984; Andelman, 1985; Cothern et al., 1986). Brown et al. (1984) recently concluded, based on an analysis of data on human dermal absorption of several organic compounds at relatively high aqueous concentrations (Dutkiewicz and Tyras, 1967, 1968), that dermal absorption of organic contaminants in drinking water constitutes the *major* uptake pathway for a variety of such compounds. Subsequent health-risk assessments, incorporating the assumptions of Brown et al. (1984) into an integrated indoor exposure model considering respiratory as well as ingestive and dermal routes of exposure, indicate that dermal absorption of VOCs from domestic water supplies may constitute a significant fraction (from 20% to 35%) of total uptake of several suspect-carcinogen VOCs such as chloroform, trichloroethylene, and tetrachloroethylene (Shehata, 1985; McKone, 1987; Bogen et al., 1988; CDHS, 1988a, 1988b). In contrast, predictions based on empirically unvalidated mathematical models of dermal absorption of VOCs at very low aqueous concentrations suggest that the dermal exposure route may in general (U.S. EPA, 1984, 1988; Brown and Hattis, 1989) or in particular cases (Shehata, 1985) account for only a small fraction of total exposure due to water ingestion alone.

Studies have been made of the extent and kinetics of dermal uptake of chlorinated solvents in humans exposed to pure solvents by thumb immersion (Stewart and Dodd, 1964; Hake and Stewart, 1977). To our knowledge, no data exist on dermal absorption in humans or animals of such compounds in dilute aqueous concentration. Apparently, this issue has only been addressed for toluene, styrene, xylene, and ethylbenzene (all nonchlorinated aromatic compounds) in the studies by Dutkiewicz and Tyras (1967, 1968). In these studies, an entire hand was immersed for 1 hr in a 1-L beaker containing one of the four compounds mentioned at initial

aqueous concentrations ranging from 66.5 to 600 mg/L at 23 to 25°C, uptake was determined by measuring the difference between the initial and final concentrations and (for styrene and ethylbenzene) by measurement of a urinary metabolite subsequent to exposure (Dutkiewicz and Tyras, 1967). From these data, Brown et al. (1984) calculated dermal permeability coefficients for these compounds in aqueous solution ranging from 0.0006 to 0.001 L/cm^2-hr and averaging 0.001 L/cm^2-hr. Based on the consistency of these calculated values, Brown et al. used the value of 0.001 L/cm^2-hr to extrapolate dermal uptake of these compounds in bathing humans at aqueous concentrations as low as 0.005 mg/L, i.e., at concentrations as much as four orders of magnitude lower than those at which Dutkiewicz and Tyras made their empirical determinations of dermal permeability.

In the absence of the relevant empirical data, it is simply unknown whether an average of dermal permeability constants derived (e.g., as done by Brown et al. (1984)) for various aromatic hydrocarbons at relatively high aqueous concentrations (in relation to their aqueous saturation point) adequately predicts the dermal permeability of small, halogenated aliphatic molecules in the extremely dilute aqueous concentrations at which these are typically found in aqueous environmental media. The present study was designed to begin to fill this data gap by measuring *in vivo* dermal absorption of a chlorinated VOC at extremely low aqueous concentrations.

Our study investigated trichloroethylene (TCE), a suspect human carcinogen that is a common groundwater contaminant throughout the U.S. at concentrations in the part-per-billion (ppb) range (EPA, 1985; Cothern et al., 1986; Bogen et al., 1988). Because of TCE's recently recognized potential carcinogenicity to humans (U.S. EPA, 1985, 1987), we used experimental animals to quantify percutaneous TCE absorption from aqueous solution. The hairless guinea pig was chosen as the animal model because guinea pig skin has been noted to provide (in comparison to that of other

animal species) a particularly useful approximation to human skin for the purpose of quantifying human epicutaneous permeability (Scheuplein and Blank, 1971; Wester and Maibach, 1983; Maibach and Wester, 1989). The hairless guinea pig, in particular, has recently been developed as an optimal experimental model for clinical research in dermatology. Because there is some evidence that the dermal absorption characteristics of hairy rodents may differ from those of hairless strains (Kao et al., 1988; Wade et al., 1989), the hairless guinea pig should serve as a better model for predicting dermal absorption characteristics of humans, who are relatively sparsely haired compared to normally haired rodents. Earlier studies of percutaneous absorption of chlorinated solvents in guinea pigs were all acute toxicity studies that involved administration of pure solvents to a cover glass glued to clipped back skin (Wahlberg, 1976; Kronevi et al., 1979).

The objective of this study was to determine the permeability of guinea pig skin to extremely dilute aqueous TCE. We also investigated percutaneous absorption of TCE at a relatively high aqueous concentration (in the range of the concentration levels investigated by Dutkiewicz and Tyras (1968) for other compounds discussed above). In addition, we measured the rate and total amount of urinary and fecal elimination of dermally absorbed TCE from dilute aqueous solution and compared these data to corresponding data from animals injected with similarly small amounts of TCE.

MATERIALS AND METHODS

Experimental Animals. Eleven female Crl:IAF(HA)BR hairless (but euthymic) guinea pigs (Charles River Laboratories, Chicago, IL), 8 to 20 weeks of age and weighing between 370 and 560 g, were used in a total of 15 animal experiments.

four of these animals were each used in two separate experiments. Food and water were available *ad libitum* except as noted below.

Anesthesia. Sedation was required to ensure that the animals used did not disturb the experimental apparatus during the course of dermal exposure. Sterile pentobarbital sodium at 50 mg/mL (Nembutal; Abbott Laboratories, N. Chicago, IL), diluted in an equal volume of sterile 0.9% NaCl in H_2O, was used to achieve sedation in all experimental animals, from which food and water were withheld 24 hr prior to sedation. The doses used were: 30 mg/kg body weight administered intraperitoneally 20 min prior to preparation for exposure plus 15 mg/kg administered subcutaneously between the left shoulder and the back of the neck 35 min into the exposure period (to maintain an even level of sedation until the end of the experiment). Five to 20% increases above these target doses were necessary in some dermal experiments to achieve effective sedation. In the four cases in which an animal was reused, the time between the first and second experiments was $\geq$ 3 weeks. Thus, for all animals used, hepatic enzyme-mediated metabolism was very unlikely to have been induced significantly above basal rate as a result of the small total pentobarbital dose received during the course of experimentation.

Dermal Exposure Chamber. A glass dermal exposure chamber (DEC), illustrated in Fig. 1, was fabricated (height = 15.0 cm, inner diam. = 8.4 cm), allowing easy attachment of a water- and air-tight diaphragm by means of a flexible rubber O-ring. The diaphragms used (one per experiment) consisted of a 12-cm square of latex rubber sheeting (thickness = 0.025 $\pm$ 0.003 in [64 $\pm$ 8 mm]; McMaster-Carr, Los Angeles, CA) into the center of which was cut a circular hole (diam. = 2.9 cm), backed by one to two layers of aluminum/polyester adhesive tape sections

(0.00089 mm aluminum + 0.0051 mm polyester film backed with pressure-activated acrylic adhesive; stock no A25, Lamart Corporation, Clifton, NJ) affixed to the circular area of latex to be enclosed by the O-ring, forming an aluminum tape undercoating (diam. = 9.0 to 9.5 cm) with a central circular hole of diameter approximately equal to that of a guinea pig chest.

Test Chemical. ^{14}C-radiolabeled TCE (4.1 mCi/mmol TCE @ >99% purity [0.032 mg TCE/μCi], Sigma Chemical Co., St. Louis, MO) was prepared in methanol to yield 0.028 μCi/μL of the methanol + TCE + ^{14}C-TCE solution (hereafter referred to as MT).

Experimental Procedure. Five "low-concentration" dermal exposure experiments (hereafter referred to as Exp. A1 to A5) were conducted using animals dermally exposed to extremely dilute aqueous TCE concentrations ranging from 16 to 85 ppb (see Table 1). Another five "high-concentration" experiments (A6 to A10) involved dermal exposure to 100,000 ppb aqueous TCE. Urinary and fecal excreta were collected from all dermally exposed animals until radioactivity measured in these products reached levels statistically indistinguishable from background.

A total of ten "DEC control" experiments were conducted at corresponding low TCE concentrations (Exp. C1 to C5) and high TCE concentrations (Exp. C6 to C10). These control experiments were identical to the corresponding experiments A1 to A10, except that the bottom of a glass beaker was substituted for a live animal in the DEC as explained below. The DEC control experiments were not conducted simultaneously with corresponding dermal exposure experiments; rather, a single DEC was used for all experiments conducted on different occasions.

In addition, five "positive control" experiments (Exp. P1 to P5) were conducted to determine the rate and extent of urinary and fecal excretion of injected radiolabel, to serve as a comparison for corresponding data obtained from dermally exposed animals.

Prior to each experiment, from 40 to 100 μL MT was diluted in 1.0 mL double-distilled H_2O (DDW) for use as the fluid volume introduced (FVI) into the DEC prior to each experimental exposure. The initial MT volume used varied between experiments because adjustments in this volume were made, using data from previous experiments, to ensure that the range of initial measured radiolabel concentrations (in dpm/mL sampled DEC fluid) obtained for Exp. A1 to A10 overlapped that obtained for Exp. C1 to C10.

All DEC and diaphragm materials and the stir bar were weighed dry prior to each experiment. In Exp. A1 to A10, the latex diaphragm was placed around the upper chest of the animal just below the front legs and shoulders. Sections of aluminum tape were then attached to the underside of the diaphragm as close to the chest of the animal as possible to present the minimum possible surface area of latex to the fluid subsequently placed into the DEC. The DEC, with a teflon-coated magnetic stir bar at the bottom, was filled to two-thirds capacity with DDW (Exp. A1 to A5), or with a 100,000-ppb solution of TCE in DDW (ppm) by weight (Exp. A6 to A10), preheated to 32 °C. The animal was then lowered into the DEC, the diaphragm was affixed to the chamber with one or two rubber O-rings, and any residual air bubble in the DEC was removed by syringe (through the diaphragm). The DEC was weighed at this time to allow the determination of the initial fluid capacity, V, of the DEC, taking into account a DDW density of 0.995 g/mL at 32 °C. Chamber water was continuously stirred and maintained at an average temperature of 32 °C (± 2 °C range) throughout each test period.

Prior to the beginning of a test period (at $t_{-0.5} = t_0$), samples were taken of DDW (0.5 mL, through the diaphragm to serve as "blank controls"), fluid contained in the DEC prepared and sealed as described above (0.5 mL), and IS (20 µL). At t_0, 1.0 mL of FVI was injected by syringe into the DEC through the diaphragm, such that at t_0 the DEC fluid volume V_0 was equal to V - 0.5 mL + 1.0 mL. Samples of DEC fluid were then withdrawn by syringe through a distant location in the diaphragm at 10, 15, 20, 30, 40, 50, 60, and 70 min after t_0. All transdiaphragm fluid withdrawals were done using a 500 µL gas-sampling syringe with a teflon-tipped plunger (Series A, #11-124C; Dynatech Precision Sampling Corp., Baton Rouge, LA), which was always triple-rinsed with DDW prior to each use. A separate 500 µL syringe was used for the input of FVI into the DEC at t_0.

After the last fluid sample was taken at t_0 + 70 min, the guinea pig was removed from the DEC and placed in a metabolism cage in a negative air-flow unit. In control Exp. C1 to C10, the bottom surface of a 4- to 5-cm diameter glass beaker was used in place of an animal in the DEC, but procedures used were otherwise identical to the dermal exposure experiments.

Positive Control Experiments. In Exp. P1 to P5, animals were sedated twice as in the DEC experiments and, within 15 min of the second sedative administration, were administered radiolabeled TCE either as a single intramuscular (IM) dose in the back of animal's left thigh (Exp. P1 and P2) or as a single subcutaneous (SC) dose in the animal's mid-back region slightly to the right of center (Exp. P3 to P5). The IM doses were dissolved in methanol (total volume = 0.2 mL) and the SC doses were dissolved in corn oil (total volume = 0.5 mL). In each of the IM and SC control experiments, an estimate of injected dose was obtained from separate samples of the radiolabel vehicle mixture used, taken prior and subsequent to animal injection.

Collection of Urinary and Fecal Metabolites. All urine and feces produced subsequent to exposure were collected for two to four weeks; samples were obtained periodically for radioactivity analysis until their radiolabel content was not statistically significantly different from background levels. Total urine volumes and fecal output mass were recorded for each animal during postexposure observation. In the four cases in which an animal was reused for a second experiment, no animal was observed to excrete radioactivity above background prior to reuse. Exp. C1 to C5 and A1 to A5 preceded Exp. C6 to C10 and A6 to A10, which in turn were followed by Exp. P1 to P5.

Measurement of Radiolabel. Postexposure output of radiolabel in urine and feces was measured in Exp. A1 to A5 and P1 to P5. All DDW blanks, DEC-fluid samples, DEC methanol-rinsate samples, urine samples (two 1-mL volumes/sample), diaphragm material samples (six 1-cm^2 sections each of latex and aluminum tape per experiment), and positive-control-injection samples collected were placed directly in 15 mL of Insta-Gel (Packard Instrument Co., Downers Grove, IL) or Universol (ICN Biomedicals, Inc., Irvine, CA) liquid scintillation cocktail (LSC) for quantification of ^{14}C in counts per min (cpm) in a Tri-Carb 4530 scintillation counter (Packard Instrument Co., Downers Grove, IL). (Our laboratory switched to the less toxic Universol LSC midway through our experiments for hazard control reasons. As each set of test and corresponding control samples was analyzed using the same LSC, the change in LSC is unlikely to have significantly influenced our analyses of *net* radiolabel concentration above background). All radiolabel concentrations measured in cpm were converted to equivalent values in disintegrations per min (dpm) using a calibration curve for ^{14}C based on external standards supplied by Packard Instrument Co.

Fecal radioactivity was assayed as follows: ten fecal pellets were selected from each weighed fecal collection, weighed, and then crushed by mortar and pestle; the fecal powder was then rinsed with 20 mL methanol. Two 1.0 mL aliquots of the rinsate were used to measure radiolabel as described above; measured dpm above background for each sample were multiplied by the appropriate factor to convert to estimated equivalent total dpm above background for each entire fecal collection. Fecal samples from nonexposed animals were used to establish background dpm levels by this procedure. Measurements of net radioactivity above background in urine samples were similarly normalized to each total collected volume. Urine collected from nonexposed animals was used to establish background dpm levels.

Estimation of Dermal Surface Areas. The animals used in Exp. A-1 to A-10 were not sacrificed immediately after exposure to allow for ongoing metabolism studies. Therefore, the exposed dermal surface areas of these animals were all estimated by interpolation using an allometric equation (Area in $cm^2 = aBW^b$, where BW = body wight in grams) fit by log-log least-squares optimization to experimentally determined surface areas of ten female hairless guinea pigs weighing between 360 and 760 g. The areas that were measured correspond to those areas dermally exposed in Exp. A-1 to A-10. Each surface area measurement was performed after sacrifice by weighing a cut-out secondary tracing of a primary tracing of dissected skin. The skins were each carefully flattened onto 8 1/2 × 11 in paper to perform the primary tracing. Secondary tracings on clean preweighed paper were performed using a light table.

Data Analysis. The relative percent losses of radiolabel from the DEC were small in each experiment, such that final radiolabel concentration was greater than 50% (generally 70% to 85% in animal-exposure experiments, and >90% for chamber

control experiments) of the value measured initially (at time = 10 min). Therefore, linear regression was used to model the change in radiolabel concentration over time, because there was no evidence or expectation of significant nonlinearity associated with a predicted first-order exponential decay in radiolabel concentration. Significance levels (p-values) reported are all for two-tailed T tests with df degrees of freedom.

RESULTS

The ten measured dermal surface areas (in cm^2) were adequately fit to the function aBW^b where BW = body weight in grams ($r^2 = 0.774$, $p = 0.00078$, $df = 8$). The resulting best-fit parameter values (and corresponding 95% confidence limits) are: $a = 24.5$ (9.11 to 65.9) and $b = 0.394$ (0.221 to 0.568) (see Fig. 2). A linear fit was found to be slightly (but not significantly) superior.

Radiolabel losses from the DEC in Exp. C1 to C5 and A1 to A5 are shown in Figs. 3a and 3b. The corresponding radiolabel loss data for each experiment are presented in Table 1. Corresponding permeability constants calculated from indirect information on dermal uptake (Figs. 3a and 3b) are given in Table 1. Cumulative postexposure output of radiolabel in urine and feces was measured for each animal used in Exp. A1 to A5 and P1 to P5, and these elimination data are summarized in Table 2.

Radiolabel losses from the DEC in Exp. C6 to C10 and A6 to A10 are shown in Figs. 4a and 4b. The corresponding radiolabel loss data for each experiment are presented in Table 1. Corresponding permeability constants calculated from indirect

information on dermal uptake (Figs. 4a and 4b) are given in Table 1. Postexposure elimination of radiolabel from animals in these high-concentration experiments was not measured in this study.

The data in Table 1 indicate that a small amount of ^{14}C-TCE was probably lost from the DEC during the course of each of the chamber control experiments (C1 to C10), but that the hourly loss rate was probably slightly higher in the high-concentration control experiments (C6 to C10) then in the low-concentration control experiments (C1 to C5). Data from each the low- and high-concentration animal experiments (A1 to A10) indicate hourly rates of ^{14}C-TCE loss from DEC water that significantly exceed those measured in Exp. C1 to C10.

All data on radiolabel loss during DEC experiments were analyzed, and are summarized in Figs. 3 and 4, as percent remaining in sampled DEC fluid relative to that measured in a sample taken 10 min after input of radiolabeled TCE in each experiment, where the latter amount was assigned the value of 100% in each experiment. Because the radiolabel content of the 1.0 mL of radiolabeled fluid injected into the DEC at t_0 was measured in each experiment (see Methods), it was possible to express the radiolabel concentration measured at 10 min (C_{10}) as a percentage of an estimated hypothetical radiolabel concentration arising from the input at time t_0 assuming instantaneous, ideal mixing. The average values (± stand. dev.) of the latter percentage for Exp. C1 to C5, A1 to A5, C6 to C10, and A6 to A10 were found to be 87.2 ± 7.4, 91.4 ± 3.4, 95.9 ± 3.9, and 98.0 ± 12.7 percent, respectively. These values are all significantly less than 100% ($df = 4$, $p < 0.05$); however, for both the low- and high-concentration experiments there is no significant difference between the control and experimental means ($p > 0.05$, $df = 8$).

Significant amounts of radiolabel were found to be present in samples of diaphragm material assayed. Thus, some of the total radiolabel injected into the DEC was lost into (and/or perhaps through) the diaphragm. Expressed as a mean

percentage (± stand. dev.) of the total amount of radiolabel present in DEC fluid sampled 10 min after t_0, the radiolabel content of the entire exposed portion of the diaphragm (based on the samples collected — see Methods) was calculated to be 1.6% (± 1.2%) and 0.47% (± 0.75%) for the control (C1 to C10) and dermal (A1 to A10) experiments, respectively (significantly different; $p < 0.05$, $df = 18$). Note that the approximate 3-fold ratio of these two values corresponds to the approximately 3-fold longer duration of diaphragm exposures in Exp. A1 to A10 compared to those in Exp. C1 to C10.

DISCUSSION

Our analysis of surface-area measurements indicates that the exposed areas of the medium-sized hairless guinea pigs we used can reasonably be interpolated from measured body weights. However, the empirical allometric exponent of body weight was found to be approximately 1/3 and to be clearly lower than the value of 2/3 typically used for estimating surface area for guinea pigs and other animals (Schmidt-Nielsen, 1984).

Using our experimental apparatus and procedures, we could not control the inter-experiment variability in radiolabel concentration measured in the sample withdrawn from the DEC at t_{10} as a fraction of that estimated to arise from the radiolabel input into the DEC at t_0. The most likely source, we feel, was inaccurate estimation of the input amount of radiolabel, given the well known capacity of TCE to "plate out" or adsorb onto glass containers, such as the glass sampling syringes used. It is also conceivable that small variations in TCE exposure conditions, such as TCE concentration or amount of water-to-latex contact, may have contributed to this variability. However, the linearity of the decrease in percent of radiolabel

remaining as a function of time, the consistency in linear decline among the control slopes and among the experimental slopes, and the highly significant difference between the latter and the former slopes, all strongly support the hypothesis that ^{14}C-TCE injected into our DEC quickly equilibrates (either consistently or, for as yet undetermined reasons, to a variable degree) with chamber water, glass, and diaphragm materials within a time ≤ 10 min after t_0, and that thereafter very little change in radiolabel concentration takes place during a 70-min exposure period in the absence of dermal exposure. This hypothesis is supported by the lack of a significant difference, as noted above (see Results), between control and experimental mean radiolabel concentrations at t_{10}, expressed as a percent of corresponding estimated concentration at t_0 assuming instantaneous, ideal mixing. Assuming the validity of this hypothesis, we obtained permeability coefficients from our data by subtracting control from experimental slopes obtained between 10 and 70 min after t_0, inclusive, and then dividing by our estimate of exposed dermal surface area (see Table 1).

Fairly consistent permeability coefficients were obtained for eight different hairless guinea pigs in ten different experiments involving dermal exposure to aqueous TCE concentrations that were either extremely low (from 20 to 110 ppb) or relatively high (100,000 ppb). The mean of the five permeability coefficients obtained with dermal exposures at very low TCE concentration did not differ significantly from that obtained using the much higher TCE concentration ($p > 0.05$, $df = 8$). This finding supports the assumption that the dermal uptake rate for TCE (in mg TCE absorbed/hr-cm^2 skin exposed) is strictly proportional to TCE concentration over a very large concentration range, where Fick's law of passive diffusion apparently governs dermal uptake of dilute aqueous TCE. Thus, our data support the "linearity" assumption used by previous investigators in model-based analyses of potential dermal uptake of chlorinated VOCs in dilute aqueous

concentration (Brown et al., 1984; Brown and Hattis, 1990). These values, however, are roughly three to five times lower than the average value obtained by Brown et al. (1984) based on the data of Dutkiewicz and Tyras (1967, 1968) on human dermal exposure to fairly dilute aqueous concentrations of several organic compounds. A discrepancy of this magnitude is not surprising, given that the latter study involved exposure to nonchlorinated aromatic compounds at aqueous concentrations roughly 1,000-fold higher than those of TCE used in our present study of guinea pigs. The human studies also involved exposure of a single hand, whereas our guinea pigs underwent approximate whole-body exposure. (Whole-hand permeability in humans was found to be only slightly less than that of the whole body per unit surface area in the case of exposure to certain pesticides (Maibach et al., 1971), so it is doubtful that the relatively higher permeability found in the Dutkiewicz and Tyras (1967, 1968) study was due solely to their restriction of exposed body portion to a hand.)

Our measurements of radiolabel excreted in urine and feces by dermally exposed guinea pigs clearly demonstrates that dermal absorption of extremely dilute aqueous TCE took place. Moreover, this information, combined with our estimates of dermally absorbed dose for each exposed animal in Exp. A1 to A5, indicates that 34% to 87% of the dermally absorbed dose in this experiment was ultimately eliminated as urinary and fecal products, while 47% to 77% of similar doses injected directly into the positive control animals was eliminated as such products.

The mean percent excreted (MPE) by these five dermally exposed animals does not differ significantly ($p > 0.05$, $df = 8$) from that of the five positive control animals. However, the kinetics of uptake, metabolism, and/or excretion appear to have been different between the positive control and dermally exposed animals, because it took about twice as long for the former animals to release their excreted yield of radiolabel as the latter animals (see Table 2; difference significant:

$p < 0.02$, df = 8). This suggests that skin may act for dermally absorbed TCE as a reservoir that empties only very slowly, presumably by loss through partition to lipid-soluble material in perfusing blood.

The MPE from experiments P1 and P2, which employed an intramuscular exposure route and a methanol vehicle, was significantly lower than that from experiments P3-P5, which employed a subcutaneous exposure route and a corn-oil vehicle ($p < 0.0001$, df = 3). This difference and the apparent corresponding difference in time-to-95% excretion of radiolabel both (see Table 2) suggest a difference in the kinetics of systemic absorption from skin and/or metabolism of TCE administered via these different routes and vehicles. Nevertheless, the MPE from both of the positive control subgroups does not differ significantly from that obtained in the low-concentration dermal experiments A1-A5 ($p > 0.005$; df = 5,6), due principally to the large degree of variability in the estimated excreted percent of dermally absorbed dose in the latter experiments.

The values for MPE to urinary and fecal products obtained in experiments A1-A5 and P1-P5 are similar to corresponding values obtained by Prout et al. (1985) for rats exposed to a small dose (10 mg/kg) of TCE by gavage. The rats were found to metabolize a total of approximately 96% of the administered TCE, with 78% of the applied dose being converted to urinary and fecal metabolites. Although the guinea pigs used in the present experiments were approximately twice the weight of the rats used in the study by Prout et al. (1985), size alone would not be expected to alter the observed metabolized fraction of very low doses of TCE if, for pharmacokinetic modeling purposes, the guinea pig could be expected to metabolize TCE like an oversized rat (Bogen, 1988). Thus, the Prout et al. (1985) rat observations lend further general support for our hypothesis that our DEC system provides a reasonable estimate of the TCE dose received by guinea pigs via percutaneous absorption from extremely dilute aqueous TCE solutions.

Our DEC-derived estimates of dermal TCE uptake in guinea pigs may be useful in the context of human health-risk assessment for dermal exposure to dilute aqueous TCE. For example, it might be assumed that dilute aqueous TCE is absorbed by human skin with a permeability constant of 0.0003 L/cm^2-hr, the approximate upper bound of that observed for the guinea pigs in the low-concentration experiments A1-A5. In this case, a 70-kg adult a with surface area of 18,000 cm^2 who is 80% immersed in water containing dilute TCE for a period of 20 min would be expected to absorb the same amount of TCE that would be ingested by drinking approximately 1.4 liters of that same water.

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Table 1. Percutaneous Absorption of Aqueous TCE in Hairless Guinea Pigs

Experiment	Animal identity	Animal weight (g)	Exposed surface area A (cm^2)	Initial water volume V_0 (mL)	TCE concentr. at time = 10 min[a] C_{10} (ppb)	Radiolabel loss rate[b] R (%/hr)	Permeability constant[c] K (L/cm^2-hr)
C1	-	-	-	795	37.9	2.6[d]	-
C2	-	-	-	803	16.0	0.4[d]	-
C3	-	-	-	808	68.6	1.9[d]	-
C4	-	-	-	813	84.7	1.2[e]	-
C5	-	-	-	799	25.2	1.9[e]	-
						1.6 ± 0.30[f]	
A1	1	473	278	450	88.3	17[g]	0.00025
A2	2	492	283	479	113	17[g]	0.00026
A3	3	423	266	509	59.3	16[g]	0.00027
A4	4	535	292	488	21.8	13[g]	0.00019
A5	3	488	282	521	21.3	12[g]	0.00019
							0.00023 ±17%[h]
C6	-	-	-	796	100,000	3.2[e]	-
C7	-	-	-	805	100,000	1.8[e]	-
C8	-	-	-	783	100,000	2.2[e]	-
C9	-	-	-	792	100,000	2.0[e]	-
C10	-	-	-	779	100,000	1.9[e]	-
						2.2 ± 0.39[f]	
A6	5	386	257	570	100,000	13[i]	0.00024
A7	6	451	273	574	100,000	9.8[i]	0.00016
A8	5	368	252	598	100,000	11[i]	0.00021
A9	7	411	263	567	100,000	13[i]	0.00023
A10	8	385	257	555	100,000	24[i]	0.00047
							0.00026 ±46%[h]

Footnotes to Table 1

[a] TCE concentration in parts per billion (ppb) by weight in H_2O at 32°C (with density 0.995 g/mL). In all low-concentration experiments (A1 to A5, C1 to C5), ^{14}C-TCE concentration was measured in 0.5 mL of chamber water sampled at 10 min after introduction of TCE with a specific activity of 4.10 μCi/μmol (0.0320 mg TCE/μCi, 6.538×10^{-9} μCi/mL-ppb). In all high-concentration experiments (A6-A10, C6-C10), the exposure chamber was prefilled with water containing 100,000 ppb TCE preheated to 32 °C.

[b] The loss rate given for each experiment is the negative of the slope of a linear regression fit to each corresponding data set shown in Figs. 2a and 2b.

[c] Permeability constants (K), expressed as liters of chamber water cleared of TCE by dermal absorption per cm^2 per hr of exposure, were calculated as $K = (V_0 - 2.0\ mL) \times (R - B)/(A \times 100\% \times 1000\ mL/L)$. In this formula, $(V_0 - 2.0\ mL)$ represents the approximate average volume of chamber water during the experiment accounting for withdrawn sample volumes (totaling 4.0 mL), B represents the background rate of radiolabel loss (see note g), and A, V_0, and R are defined, respectively, in columns 4, 5, and 7 of this table.

[d] Not significantly different from zero ($p > 0.05$, $5 \leq df \leq 6$).

[e] Significantly greater than zero ($p < 0.00005$, $df = 10$).

[f] Negative of the slope (± standard deviation) of the regression line fit to all corresponding chamber control data (C1-C5 or C6-C10); both significantly greater than zero ($p < 10^{-5}$, with $r = -0.637$ and $df = 45$ and with $r = -0.604$ and $df = 58$ for the low and high concentration groups, respectively). This slope for Exp. C6 to C10 is significantly greater than that for Exp. C1 to C5 ($p < 10^{-6}$, $df = 103$).

[g] Significantly greater than the pooled control value of 1.6 %/hr ($p < 10^{-6}$, $df = 51$).

[h] Arithmetic mean ± standard deviation (as a percentage of mean).

[i] Significantly greater than the pooled control value of 2.2%/hr ($10^{-9} < p < 0.025$, $df = 64$).

Table 2.
Excretion of Radiolabel in Urine and Feces
by Hairless Guinea Pigs Exposed to ^{14}C-TCE

Experiment	Animal identity	Exposure regimen[a]	Administered dose[b] (μg TCE)	Total radiolabel excreted[c] (μg TCE-equiv.)	Excreted percent of administered dose (%)	Fecal percent of total excreted dose[d] (%)	Time to excrete 95% of metabolized dose (days)
		T_e (min)					
A1	1	Dermal (85)	8.8	5.57	63	3.0	11
A2	2	Dermal (75)	11	5.00	45	7.1	6
A3	3	Dermal (72)	5.3[e]	3.03	57	20	5
A4	4	Dermal (72)	1.5	1.22	81	48	7
A5	3	Dermal (72)	1.4	0.482	34	28	14
					56 ± 18[f]		9 ± 4[f]
		Animal wt. (g)					
P1	1	IM 467	40.6	19.8	49	13	8
P2	2	IM 550	20.4	9.60	47	16	17
P3	9	SC 469	2.60	1.99	76	52	22
P4	10	SC 436	2.66	2.02	76	40	21
P5	11	SC 561	2.71	2.08	77	39	22
					65 ± 16[f]		18 ± 6[f]

[a] Animals in Exp. A1 to A5 were placed in the dermal exposure chamber for a total of T_e min; positive control animals received ^{14}C-TCE (4.10 μCi/μmol) by either intramuscular (IM) or subcutaneous (SC) administration (see Methods). Weights of the animals used in Exp. A1 to A5 appear in Table 1.

[b] Total excreted radiolabel refers here to net radiolabel measured in urine, feces and cage rinse (which was generally negligible) only, in excess of background (see

Methods). Administered doses in μg for animals used in Exp. A1 to A5 were estimated as

$$K \times \frac{T_e}{60\ \text{min/hr}} \times \left(1 + \frac{R \times 10\ \text{min}}{100\% \times 60\ \text{min/hr}}\right) C_{10} \times 0.995 \frac{\mu g}{\text{L-ppb@32°C}} \times A$$

in which the definitions and corresponding values of K, T_e, R, C_{10}, and A are given in Table 1. Administered doses for positive control animals were estimated from the amount of radiolabel measured in samples of administered material (see Methods).

[c] Excreted radiolabel in urine and feces is expected to be associated solely with TCE metabolites (e.g., trichloroacetic acid and free and conjugated trichloroethanol) normally produced in rodents and not with the volatile parent compound (see U.S. EPA, 1985; Bogen et al., 1988).

[d] Some of the measured activity associated with fecal material was probably due to radiolabel in urine deposited on fecal material prior to fecal collections for sampling.

[e] Includes 0.23 μg TCE found in cage rinse, which was assigned to urine and feces in proportion to amounts actually found in urinary and fecal output.

[f] Arithmetic mean ± standard deviation.

FIGURE LEGENDS

Figure 1. Dermal exposure chamber for guinea pigs (capacity = 0.86 L with no animal present). The sedated animal was placed through a hole in the latex diaphragm, which was then undercoated with aluminum-faced tape. Once attached to the chamber, the diaphragm maintains an air- and water-tight seal around the animal allowing an aqueous dermal exposure with no airspace.

Figure 2. Measured dermal areas of ten female hairless guinea pigs vs. body weight (data points), with areas corresponding to those exposed in Exp. A1 - A10, fit to the allometric regression equation: Area (cm^2) = 24.5[Body Weight (g)]$^{0.394}$ (solid curve).

Figure 3. Amount of radiolabel remaining in the dermal exposure chamber water relative to that measured 10 min after radiolabel input, at which time the aqueous TCE concentration was approximately 20 to 110 ppb (see Table 1). **(a)** Five control experiments, three lasting 70 min and two lasting 190 min; the linear regression line for the combined data is overlaid on the individual data points from each experiment at each sampling time. **(b)** Five dermal exposure experiments lasting approximately 70 min; linear regression lines are shown for data from each experiment, overlaid on points representing arithmetic mean (± stand. dev.) of the five experimental values measured for each sampling time.

Figure 4. Amount of radiolabel remaining in dermal exposure chamber water relative to that measured 10 min after radiolabel input, at which time the aqueous

TCE concentration was approximately 100,000 ppb (see Table 1). (a) Five control experiments lasting 190 min; the linear regression line for the combined data is overlaid on points representing arithmetic means (± stand. dev.) of the five experimental values at each sampling time. (b) Five dermal exposure experiments lasting approximately 70 min; linear regression lines are shown for data from each experiment, overlaid on points representing the five experimental values measured for each sampling time.

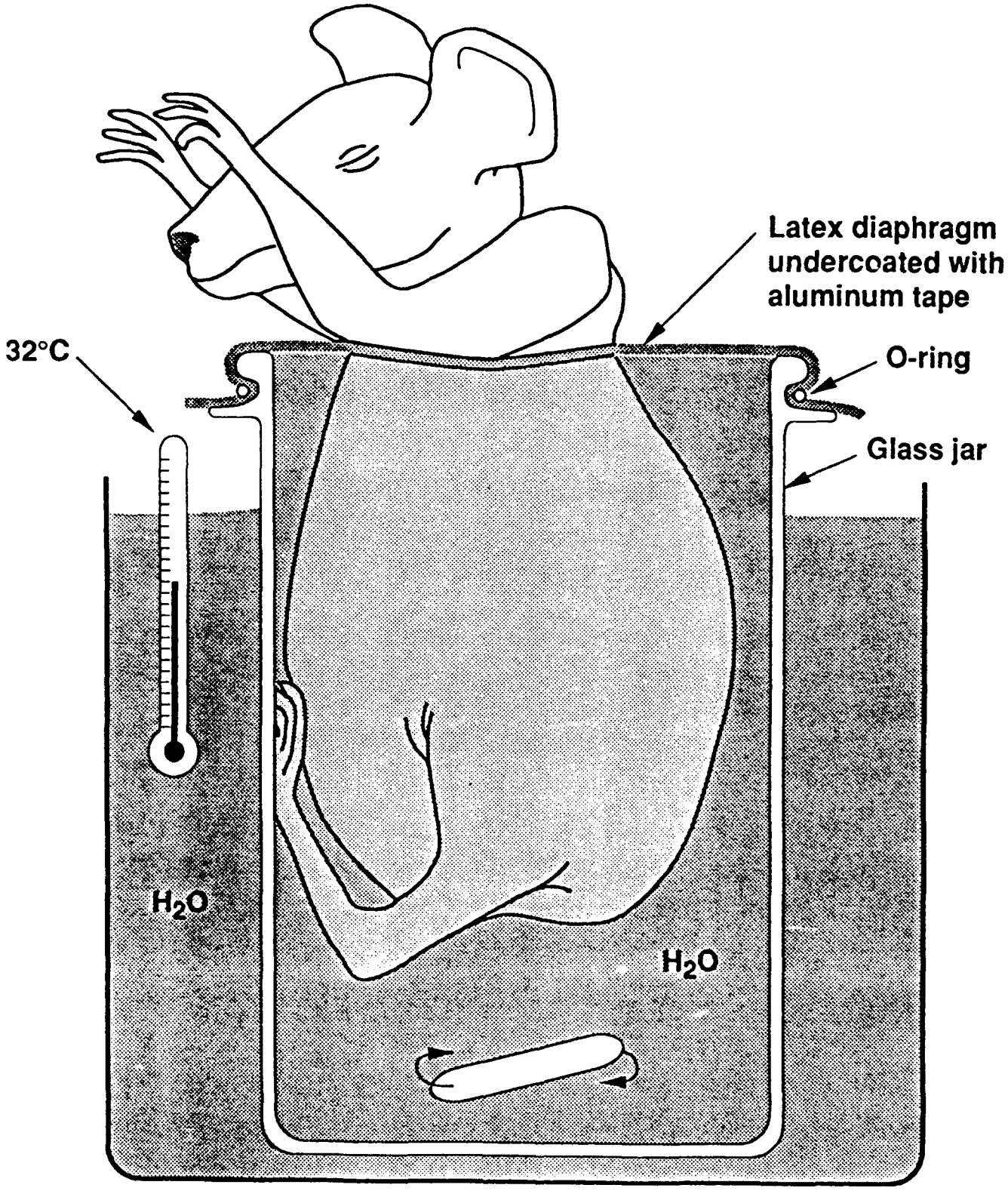
Latex diaphragm
undercoated with
aluminum tape
32°C
O-ring
Glass jar
H_2O
H_2O

Figure 1

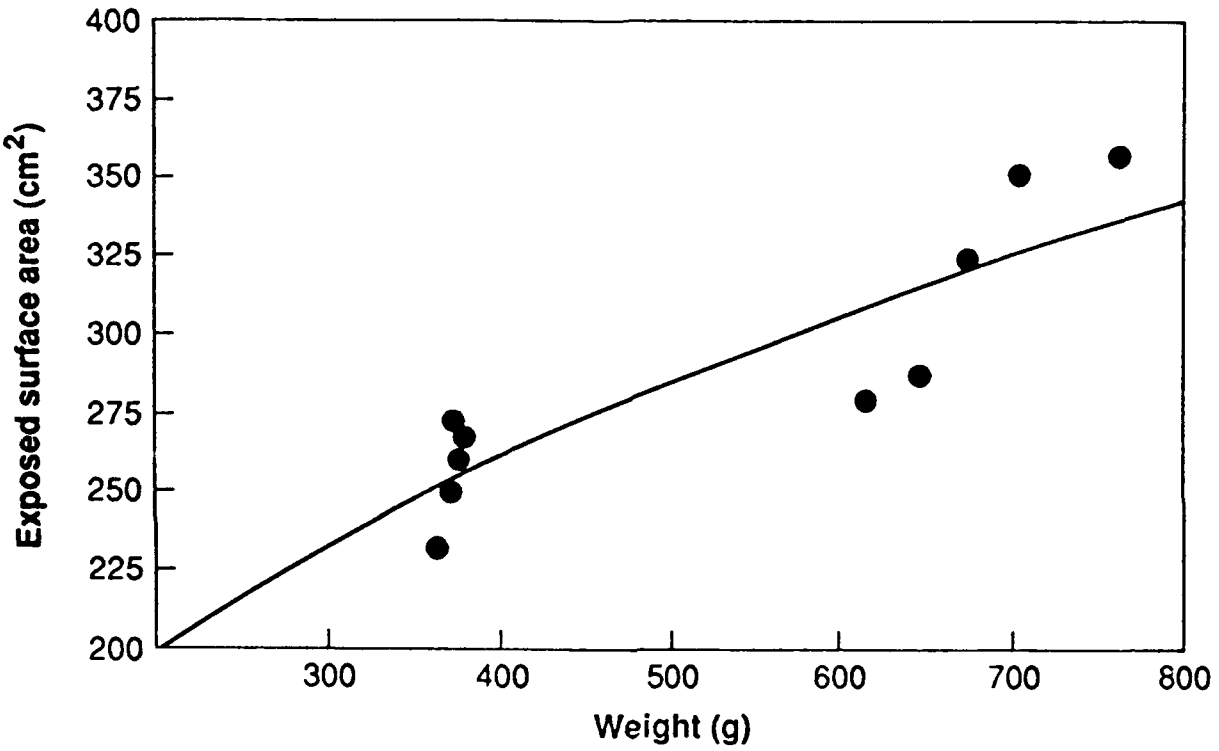
400
375
350
325
300
275
250
225
200
Exposed surface area (cm²)
300
400
500
600
700
800
Weight (g)

Figure 2

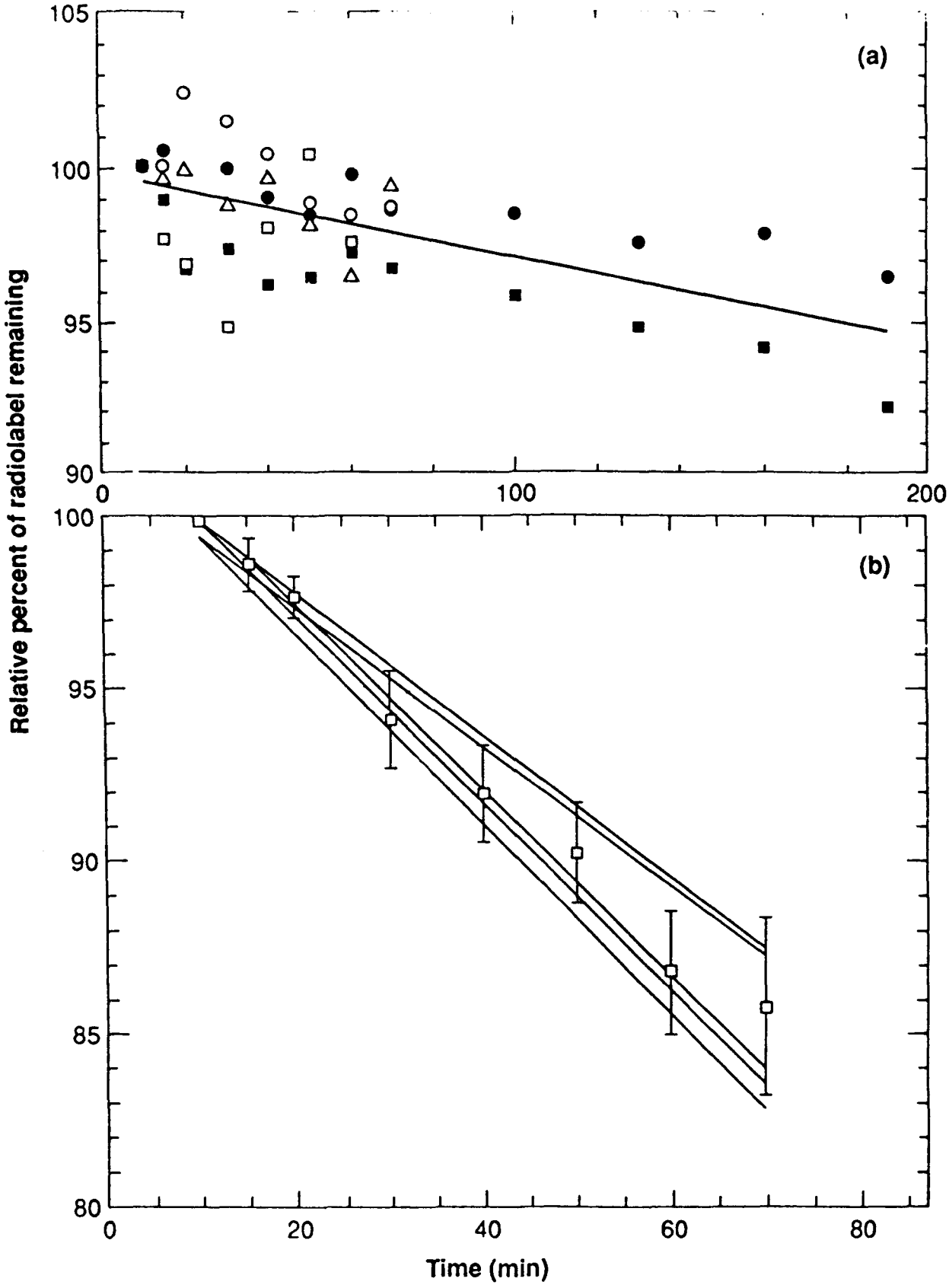
(a)
(b)
105
100
95
90
0
100
200
100
95
90
85
80
0
20
40
60
80
Relative percent of radiolabel remaining
Time (min)

Figure 3

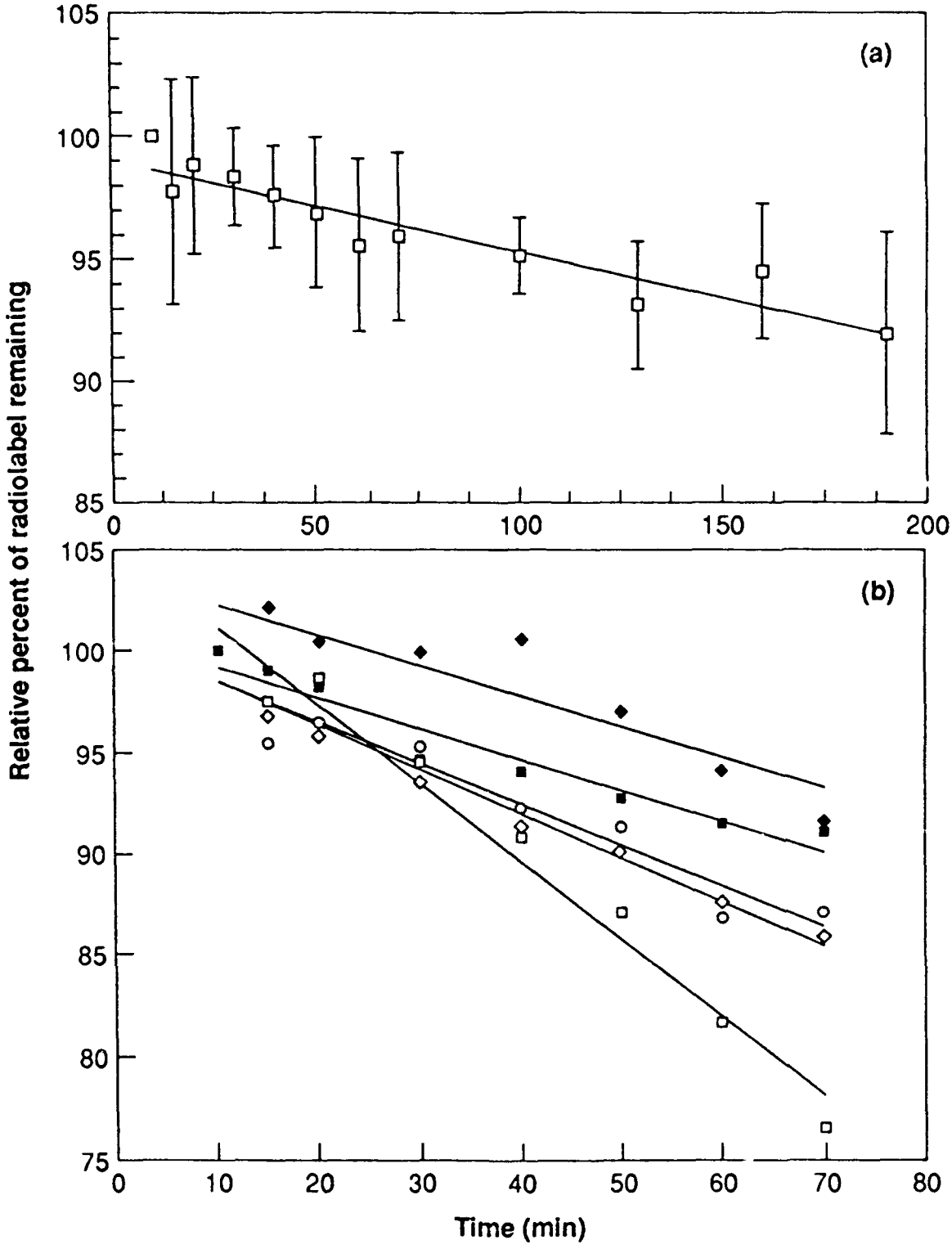

Figure 4

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